

Method validation for determination of metformin hydrochloride in pharmaceutical formulations by capillary electrophoresis with capacitively coupled contactless conductivity detection

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Abstract A method for the determination of metformin hydrochloride (MH) in pharmaceutical formulations by capillary electrophoresis with capacitively coupled contactless conductivity (C⁴D) detection was investigated. The separation was achieved under normal polarity mode at 17.5 °C, 30 kV, hydrodynamic injection (50 mbar for 8 s) and using a bare fused silica capillary 72 cm × 75 μm i.d. (detection length, 10.5 cm from the outlet end of the capillary). The optimized background electrolyte consisted of 10 mM 2-morpholinoethanesulfonic acid and 10 mM histidine, pH 6.8. C⁴D parameters were set at fixed amplitude of 100 V and frequency of 650 kHz. Under the optimum conditions, the method shows good linearity over the range of 10-30 μg mL⁻¹ MH ($r^2=0.9971$). Limits of detection and quantitation based on S/N ratio of 3 and 10 were 0.049 and 0.15 μg mL⁻¹, respectively. The proposed method was successfully applied to the assay of MH in pharmaceutical formulations and establishing the dissolution profiles for both immediate and extended release formulations of MH.

Keywords: CE-C⁴D; Metformin hydrochloride; Drug product; Dissolution profile.

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Metformin hydrochloride (MH) (N, N-dimethylimidodicarbonimidic diamide hydrochloride (Fig. 1 (a)) is recommended as an antihyperglycemic drug that stimulates glycolysis in peripheral tissues [1]. It is an oral biguanide drug that is formulated as tablet dosage form. This drug, either alone or in combination with other drugs, is currently one of the mainstays in the management of type 2 diabetes. It is often recommended for obese patients as it does not cause weight gain and has been shown to possess lipid-lowering properties [2, 3]. It can increase insulin sensitivity, improves glucose tolerance and reduces hepatic glucose production [4].

Several methods have been published for the analysis of MH in pharmaceutical preparations, human plasma, urine, breast milk, dietary supplements and herbal medicines, etc. Gas chromatography (GC) either with mass spectrometry (MS) [5] or flame ionization (FID) [6] have been reported. As MH is nonvolatile, derivatization procedure (which are tedious and time consuming) is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column. High performance liquid chromatography (HPLC) coupled with ultraviolet (UV) absorption [7-18], fluorescence [19] and MS detection [4, 20-25] are currently the most commonly used methods for the analysis of MH. Generally, LC methods provide satisfactory quantification together with acceptable sensitivity. However, LC methods in general require long analysis time and consume large amounts of solvent.

A few capillary electrophoresis (CE) methods have also been reported for the determination of MH in pharmaceutical products blood plasma and urine [26-35]. The main advantages of CE

over HPLC are the higher separation efficiency, reduced sample and reagent consumption and shorter analysis time. The combination of CE with capacitively coupled contactless conductivity detection (C⁴D) is an attractive analytical technique that has been employed for numerous applications, including pharmaceutical analysis. C⁴D can be considered as a general detection technique which shows good sensitivity for ionic species [36-38]. The popularity of C⁴D detector has been growing due to its robustness, minimal maintenance demands and low cost [39]. There are a number of recent general review articles that discussed applications of C⁴D in CE [40-43].

The main aim of this work was to develop and validate a CE-C⁴D method for the determination of MH. The method will be used for the determination of MH in formulations and also to evaluate the dissolution profiles of drug products which are normally achieved using UV spectrophotometry or HPLC methods. Pseudoephedrine HCl (Fig. 1 (b)) was used as an internal standard (IS).

Experimental

Chemicals and reagents

Metformin HCl (MH) and pseudoephedrine HCl (PEH) were kindly donated by Hikma Pharmaceuticals (Amman, Jordan). 2 (N-Morpholino) ethanesulfonic acid monohydrate (MES) (99%), potassium phosphate monobasic ($\geq 99.0\%$) and sodium hydroxide ($\geq 98.0\%$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). L-histidine (His) (98%) was

purchased from Merck (Darmstadt, Germany). Commercial pharmaceutical preparation in the form of tablets was purchased from a local pharmacy store. Ultrapure water (resistivity, $18.2 \text{ M}\Omega \text{ cm}^{-1}$) was produced by a Milli-Q system (Millipore, MA, USA) and was used throughout for the preparation of solutions. The pH of solutions were determined with a pH-meter (Orion pH meter model EA 940 (Orion Research, Cambridge, USA)). All standards, samples, background electrolyte (BGE) and NaOH solutions were filtered through $0.45 \mu\text{m}$ nylon filter membranes (Agilent Technologies, Waldbronn, Germany).

Instrumentation and electrophoretic conditions

A HP^{3D}CE capillary zone electrophoresis system, model 7100 (Agilent Technologies, Waldbronn, Germany) equipped with C⁴D (eDAQ, Denistone East, Australia) was used. A bare fused-silica capillary $72 \text{ cm} \times 75 \mu\text{m}$ i.d. (detection length, 10.5 cm from the outlet end of the capillary) equipped by Agilent Technologies (Waldbronn, Germany) was employed. The new capillary was conditioned by flushing with 1.0 M NaOH (15 m), followed by 0.1 M NaOH (10 m) and then with water (10 m) before rinsing with BGE solution (15 m). Before the analysis, the capillary was rinsed for 5 m with NaOH (0.1 M), 5 m with water and 5 m with BGE. Between injections, the capillary was preconditioned with NaOH (0.1 M), water and followed by BGE solution (each for 3 m). After the last analysis, the capillary was flushed for 3 m with NaOH (0.1 M) and 3 m with water. Injection of standards and samples were carried out hydrodynamically at 50 mbar for 8 s under the following conditions: voltage, 30 kV (normal polarity); capillary temperature, $17.5 \text{ }^\circ\text{C}$; and BGE, 10 mM (MES) and 10 mM (His) pH 6.8. The pH of BGE was

adjusted with small amounts of NaOH solution. The data was obtained by using PowerChrom software version 2.6.11 (eDAQ, Denistone East, Australia). The dissolution profiles of MH tablets were carried out using Pharma Test dissolution apparatus (Hainburg, Germany).

Preparation of standards and BGE

Stock solutions of MH ($1000 \mu\text{g mL}^{-1}$) and PEH ($500 \mu\text{g mL}^{-1}$) were dissolved in water. These solutions were stored at 4°C until the analysis. The BGE solution was prepared from stock solutions of 100 mM MES and 100 mM His. It was filtered through a $0.45 \mu\text{m}$ membrane filter and degassed for 15 m before use.

Sample preparation and analysis procedure for assay test and dissolution profiles

Twenty tablets of each product were individually weighed, ground and mixed in a mortar to a fine powder. A 100 mg of the powder was taken and dissolved in water, sonicated using ultrasonic water bath **elmasonic, model S 80H (Elma, Schmidbauer GmbH, Singen, Germany)** for 15 m and then diluted to 100 mL with water. 1 mL of the solution was diluted to 50 mL after spiking with the internal standard (final concentration, $15 \mu\text{g mL}^{-1}$). The sample was filtered through $0.45 \mu\text{m}$ nylon filter membranes and was introduced to the CE-C⁴D for the analysis.

The dissolution profiles of the MH tablets were determined as specified in the United States Pharmacopeia (USP) [44]. Three drug products (**A (Glucophage 500 mg, Merck) as immediate release, B (HovidEuform XR 500 mg, Hovid Berhad) and C (Metformin Hydrochloride ER 850**

mg, Cipla) as extended release, 6 tablets per manufacturer) were evaluated. One tablet was added in each one of the six glasses using 1000 mL of freshly phosphate prepared buffer (pH = 6.8) as dissolution medium. The bath temperature was operated at 37 ± 0.5 °C. Paddle dissolution apparatus was used at stirring speed of 50 rpm for product A and 100 rpm for products B and C. Sampling for the product A was done at 10, 20, 30, 45 and 60 m while for products B and C were at 1, 2, 3, 6 and 10 h. The solutions were filtered through 0.45 μ m nylon filter membranes, diluted in water and then transferred to the CE vials after spiking with IS.

Results and discussion

Optimization of C⁴D and instrumental conditions

The selection of BGE constituents is important and it should have low conductivity that will lead to small joule heating [45]. Mixtures of MES and His was used to ensure that the background conductivity is as low as possible. As MH is a strongly basic compound (pKa = 12.4) [16], it is positively charged under acidic conditions. Initially, the pH of the MES/His buffer was 6.1. Since MH is not completely deprotonated at pH 6.1, small amounts of NaOH solution were added in order to increase its pH. The BGE pH was therefore varied from 6.4-7.0. Good separation of MH with good peak shape and sensitivity was obtained when the BGE pH was 6.8. Therefore, it was selected for further studies. The effect of the concentration of MES/His buffer (5-15 mM) on the separation was also investigated. The peak was found to be broadened as the concentration of buffer is increased. 10 mM MES/His was selected as it gave the highest response and symmetrical peaks.

The detector should be operated at its optimum frequency, which is as low as possible to reduce the impact of stray capacitance [46]. The frequency was studied from 550 to 700 kHz. The highest signal and more stable baseline were found when 650 kHz used at 2V (Fig. 2). The effect of applied voltage (25–30 kV) on the migration time and separation were also studied. When 30 kV was used, sharper peaks with shorter analysis time were obtained. Therefore, 30 kV was selected for the subsequent work. The effect of capillary temperature (15-25°C) was also investigated. Best peak shapes and stable baselines were obtained at 17.5°C. Optimization of sample injection time (3–9 s) at 50 mbar was conducted to achieve a lower detection limit without affecting the quality of the peak shape, reproducibility and migration time. An injection time of 8 s offered the best results and was selected for the rest of the studies.

The selected conditions are summarized in Table 1 while Fig. 3 shows typical electropherogram. It is clear that the selected electrophoretic conditions provide good quality peaks. Table 2 summarizes a comparison of the important parameters of the present method and those reported in the literature.

Validation of analytical method

Linearity

Under the optimized conditions, linearity of the calibration plots was studied using standard MH to cover concentrations levels (10-30 $\mu\text{g mL}^{-1}$) with PEH (15 $\mu\text{g mL}^{-1}$) as IS. Calibration curve was established by plotting the corrected peak area (y) versus MH

concentration (x) in $\mu\text{g mL}^{-1}$. Calibration curves were linear over the studied concentration range. The regression equation and correlation coefficient were: $y = 0.0522 x - 0.0543$ ($r^2 = 0.997$).

Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated based on the signal-to-noise ratio (S/N) of 3 and 10, respectively. The obtained LOD and LOQ values were 0.049 and 0.15 $\mu\text{g mL}^{-1}$, respectively. These values are lower than the previously reported CE methods using UV or DAD detectors but higher than the electrochemiluminescence with LLE and derivatization, mass spectrometry, C^4D with large volume sample stacking and UV with Solid-phase extraction detectors (Table 2).

Precision

Intra-day precision was evaluated by introducing three different preparations of the standard solutions at three concentration levels (10, 20 and 30 $\mu\text{g mL}^{-1}$) on the same day ($n = 9$). Each concentration was injected three times, while the inter-day precision was evaluated over six consecutive days ($n = 54$). The relative standard deviation (RSD%) values for migration time and corrected peak area were less than 1.2 and 7.5%, respectively. The results are displayed in Table 3, demonstrating the good repeatability of the method.

Accuracy

The accuracy of the proposed method was determined by conducting recovery experiments at three different concentrations (10, 20 and 30 $\mu\text{g mL}^{-1}$). Recoveries were performed by adding known amounts of MH and IS to the analytical placebo solution. Each

concentration level was prepared in triplicates and introduced to the CE-C⁴D thrice. The results obtained are summarized in Table 4. The good recoveries obtained indicate the potential of this method for the determination of the MH in pharmaceutical formulations.

Assay and dissolution profiles of MH in pharmaceutical preparations

The validated CE-C⁴D method was applied for the assay of three commercial products (A, B and C) that have different amounts of MH. Each product was prepared twice and introduced thrice to the CE-C⁴D system for the separation. Good agreement between the total value as claimed by the manufacturer and the developed CE-C⁴D method was obtained (Table 5).

Dissolution profiles of each product were also established. The drug released was plotted against time. Product A maintained a dissolution rate of > 80% by the end of the 30 **m**. The USP requires only one time point (30 **m**) to be used for the analysis immediate release tablet. However, in the present studies, five data points (10, 20, 30, 45 and 60 **m**) were obtained. The dissolution rate for product B and C were > 85% by the end of 10 h. As shown in Fig. 4 (A and B) the dissolution profiles of all drug products met the USP specifications at each time point.

2 Conclusion

The determination of MH in pharmaceutical formulations was successfully developed and optimized using CE-C⁴D method. The simplicity (no need for derivatization), good sensitivity (LOQ value 0.15 µg mL⁻¹), rapidity, good accuracy and low cost (low consumption of

reagents and samples) are the major advantages of this method. The assay method was applied to determine the content of MH in three commercial products. The usefulness of described method is proven by successful application for the analysis of MH in tablet formulations and establishing dissolution profiles of different MH tablets, thus lending itself as an interesting alternative analytical tool for quality control laboratories.

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Figure captions

Fig. 1. Chemical structures of (a) MH and (b) pseudoephedrine hydrochloride (internal standard) used in the study.

Fig. 2. Effect of excitation frequency (kHz) on peak area. CE-C⁴D conditions: BGE, 10 mM MES/His (pH6.8); applied voltage, 30 kV; capillary temperature, 25 °C.

Fig. 3. Electropherograms of standard MH (100 µg mL⁻¹) under the optimum CE conditions (Table 1).

Fig. 4. Dissolution profiles of (A) immediate release and (B) extended release formulations. Refer to text for experimental conditions.

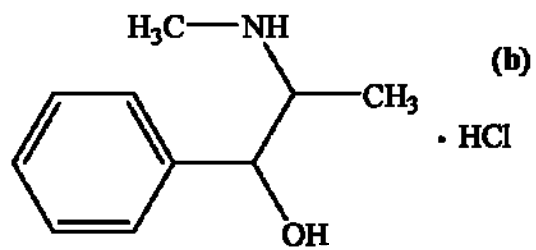
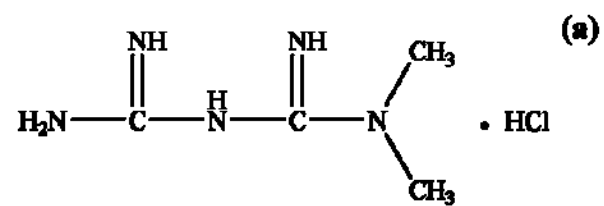


Fig. 1.

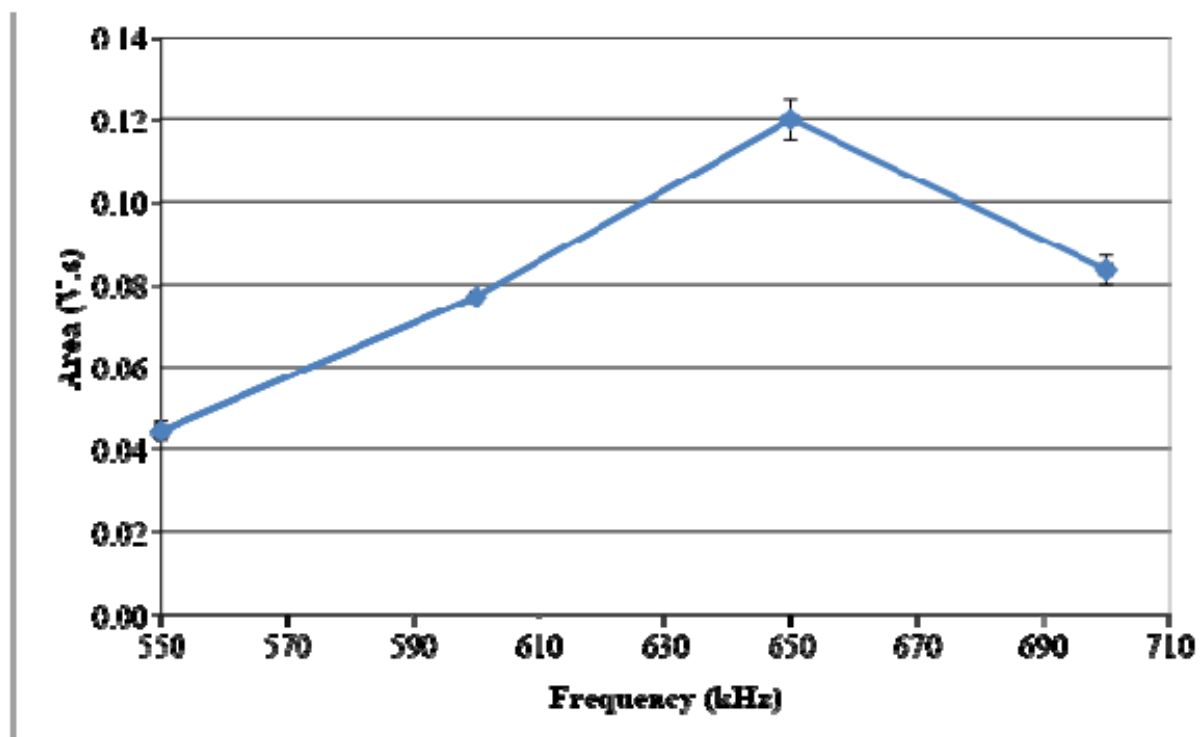


Fig. 2.

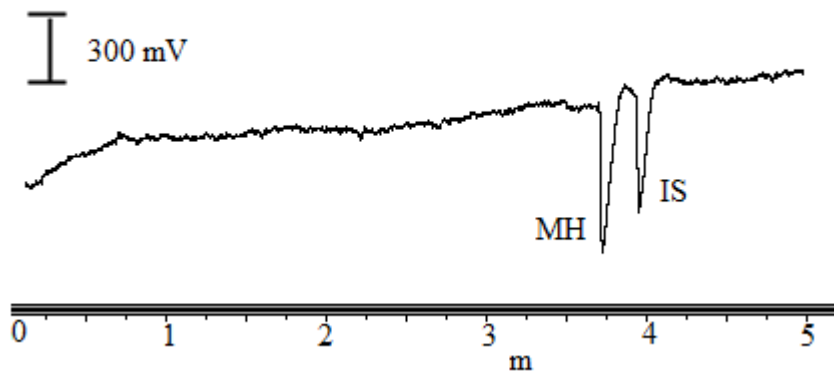


Fig. 3.

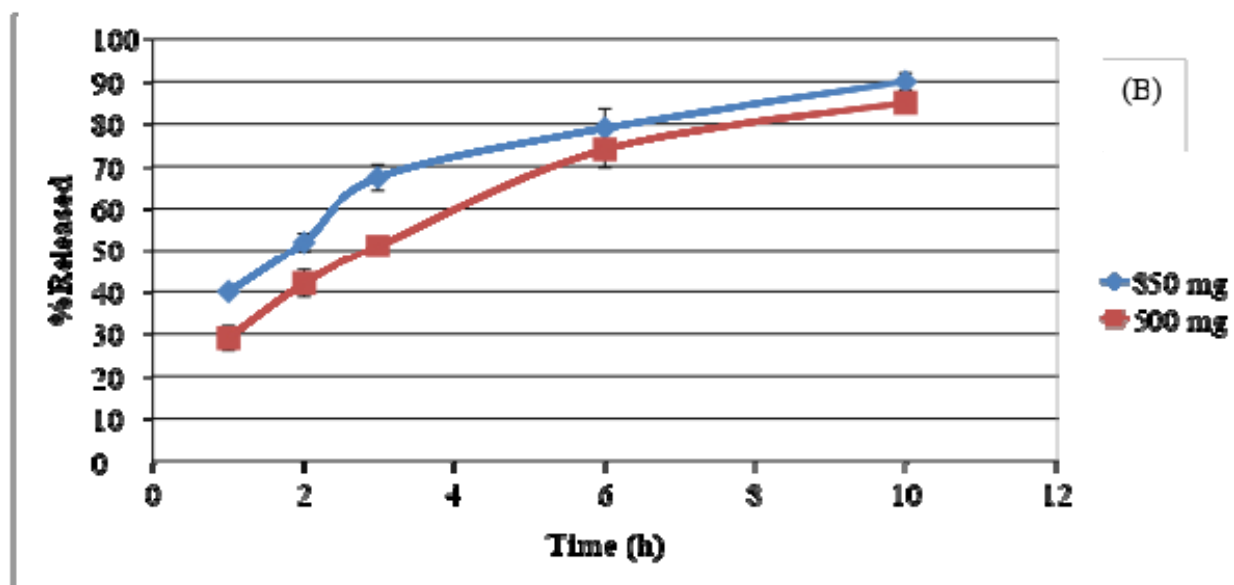
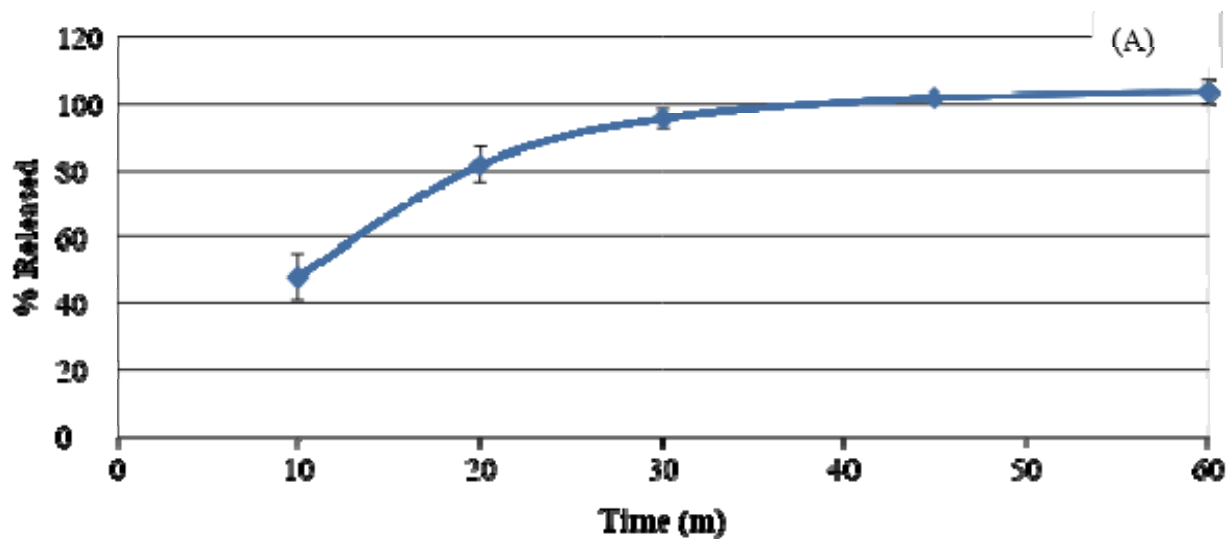


Fig. 4.

Table 1Optimum CE-C⁴D operating conditions

Background electrolyte	10 mM MES/His, pH 6.8
Applied voltage	30 kV (normal polarity)
Injection time	8 s hydrodynamic (50 mbar)
Capillary temperature	17.5°C
Bare fused silica capillary	72 cm × 75 μm i.d (detection length, 10.5 cm from the outlet end of the capillary)
C ⁴ D detector configuration	Amplitude, 100 V; frequency, 650 kHz

Table 2. Comparison of the proposed CE-C⁴D method with other reported methods for the determination of MH

Instrumentation	Detection	Sample preparation	Type of sample	Migration/ Retention time (m)	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)	Repeatability (%RSD)	Rec (%)
CE ^a	C ⁴ D ^b	Dilution	Tablet	3.7	49	150	≤7.5	96.5
CE	C ⁴ D	—	Herbal formulations	4.8	3200	9730	≤1.04	96.2
CE	MS ^d	PP ^e	Serum	8.5	2.14	7.06	≤ 3.4	85.5
CE	ECL ^f	LLE ^g + Driv ^h	Urine	3.1	0.31	1	≤ 1.7	98.5
CE	UV ⁱ	FASS ^j	Plasma	4	100	250	≤12.4	58.9
Non-aqueous CE	UV	SPE ^k	Plasma	1.75	12	—	—	—
CE	UV	Dilution	Tablet	6.2	500	1000	≤1.78	102
CE	UV	—	Plasma	2.5	30	100	≤1.7	97.3
CE	UV	Dilution	Tablet	5	830	2500	≤2.9	97.6
CE	DAD ^l	Dilution	Tablet	5.0	2000	8000	≤1.72	99.9
CE	DAD	Dilution	Tablet	9.79	30×10 ³	100×10 ³	≤1.77	98.3
GC	MS	PP	Plasma	5.5	40	100	≤ 15	69.8
GC ⁱ	FID ^j	Driv +Dilution	Tablet	7.0	0.025	0.075	≤3.9	96.3
LC ^k	MS/MS ^l	PP ^m	Plasma	1.2	—	0.02	≤15	77-9
LC	UV	PP	Plasma	7.5	—	0.03	≤11.28	87.3
LC	UV	PP	Plasma	5.0	0.100	0.250	≤5.65	91.4
LC	PDA ⁿ	Dry blood spot	Blood	5.5	0.09	0.15	≤15	84.0
LC	UV	Dilution	Tablet	10.59	0.1	0.350	—	99.8
LC	UV	Dilution	Tablet	5.04	0.023	0.069	1.01	99.4

^a Capillary electrophoresis

^b Capacitively coupled contactless conductivity detection

- ^c Large volume sample stacking
- ^d Mass spectrometry
- ^e Protein Precipitation
- ^f Electrochemiluminescence
- ^g Liquid–liquid extraction
- ^h Derivatization
- ⁱ Ultraviolet detection
- ^j Field-amplified sample stacking
- ^k Solid-phase extraction
- ^l Diode array detection
- ^m Gas chromatography
- ⁿ Flame ionization detector
- ^o Liquid chromatography
- ^p Tandem mass spectrometry
- ^q photodiode array detector

Table 3. Intra-day and inter-day precision (%RSD) of the MH standard solution using CE-C⁴D

MH concentration($\mu\text{g mL}^{-1}$)	Relative standard deviation, RSD (%)	
	Migration time	Corrected peak area
Intra-day precision(n=9)		
10	0.4	6.3
20	0.2	6.7
30	0.3	6.9
Inter-day precision(n=54)		
10	1.2	6.6
20	1.2	7.5
30	1.3	5.8

Table 4. Recoveries obtained from the determination of MH in placebos

$\mu\text{g mL}^{-1}$	% Recovery \pm SD (n = 9)		
	A	B	C
10	101 \pm 1.2	101 \pm 0.58	100 \pm 0.81
20	100 \pm 1.1	100 \pm 1.6	99.4 \pm 2.4
30	96.5 \pm 1.9	97.5 \pm 2.6	99.6 \pm 2.1

Table 5: Assay results of MH in different pharmaceutical formulation.

Product	Label claim (mg)	%Agreement \pm SD
A	500	99.8 \pm 3.4
B	500	99.4 \pm 3.1
C	850	101 \pm 2.4